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Induction of Autoantibodies to Human Enzymes Following Viral Infection: A Biologically Relevant Hypothesis

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Summary: Macro enzymes, i. e. complexes of normal (iso-)enzymes with an immunoglobulin, may be due to immunological cross-reactions evoked by specific viral antigenic determinants that are homologous to regions in the target enzymes. A search of the National Biomedical Research Foundation protein databank with the amino-acid sequence of human pancreatic amylase revealed a marked homology with a fragment of the yellow fever virus major envelope protein E: i. e. an overall identity of 19.7 per cent and a high degree (40.9 per cent) of conservative amino-acid substitutions over 119 amino acids. At each identical position, the corresponding residue of Taka amylase A was examined by three-dimensional structure analysis, to determine whether the position is likely to be buried or exposed. The existence of a site (epitope) on amylase recognized by an anti-amylase antibody is discussed.

Introduction

Macro enzymes are enzymes in serum with a higher relative molecular mass (M_r) than the corresponding enzyme normally present in serum under (patho)physiological conditions. Macro enzymes are usually complexes of normal (iso-)enzymes with an immunoglobulin. To be classified as a true macro enzyme, a protein complex must satisfy certain criteria. First, a subunit of the complex should be an immunoglobulin. Second, the binding of the antigen to the immunoglobulin should take place by the formation of non-covalent bounds between the antigen and the antigen-binding region of the immunoglobulin. Third, the antigen-antibody reaction should be reversible. Consequently, the law of mass action from which the antibody affinity can be calculated is relevant. Finally, the antigen-antibody reaction should demonstrate specificity and a high level of affinity (1). Of all the reported groups of immunoglobulin-bound macro enzymes (2, 3) to date, only macro amylase (EC 3.2.1.1), macro lactate dehydrogenase (EC 1.1.1.27), and macro creatine kinase (EC 2.7.3.2) type 1 fulfill the above criteria.

A characteristic feature of individuals suffering from one of these three macro enzymes is the persistent presence of the complex during the entire follow-up period. This indicates an underlying chronic event which in relation to the high prevalence of light chain restriction is initiated and continued by a pathogenic factor (4).

This raises the question of how these individuals are stimulated to mount a strong chronic immune response to the enzyme antigen. By molecular mimicry, an immune response to certain viral antigens may produce antibodies that cross-react with normal host antigens (5, 6); i. e. amylase, lactate dehydrogenase, and creatine kinase. Chemical similarity of amino-

acid residues is a necessary, but not a sufficient, criterion for antigenic similarity: secondary and tertiary structures also make an important contribution to the identities of epitopes. At this stage, information on three-dimensional structure is available only for amylase (7, 8). The current paper therefore describes the results of a computer search, in which the sequence of human pancreatic amylase was compared with proteins of viruses known to infect humans. The corresponding positions with identity between residues were determined in the three-dimensional structure of Taka amylase A.

Methods

The amino-acid sequences of Taka amylase A (9) and porcine pancreatic amylase (10) were aligned using available structural information. The alignments were generated using the Brookhaven program BESTFIT (11). Taka amylase A coordinates (7) from the protein databank entry 2TAA (12) were analysed with the program DSSP (13) to obtain positions of secondary structural elements. Secondary structural elements of porcine pancreatic amylase were assigned according to the study of *Buisson* et al. (8). Sequences of human pancreatic amylase (14) and porcine pancreatic amylase were aligned without deletions or insertions.

Sequence comparisons between human pancreatic amylase and the proteins in the NBRF protein databank (15) were performed using the program FASTA (16). The statistical significance of the alignments were assessed using a modification of the program BESTFIT (11).

At positions where human pancreatic amylase and the yellow fever virus major envelope protein E (17) were found to be identical, the corresponding residue of Taka amylase A was examined to determine whether the position is likely to be buried or exposed. The structural analysis was performed with the program FRODO (18).

The stereo drawings of the α-carbon backbone of the $(\alpha/\beta)_8$ barrel domain of Taka amylase A were generated using the program ARTIPLOT (19).

Results

The availability of computer programs (11), designed to search for homologous sequences, allowed us to explore the possibility that at least one viral peptide segment is sufficiently similar to a region in the target human pancreatic amylase. As a result the NBRF protein databank containing 13 413 sequences with a total of 3 707 414 residues showed an apparent sequence homology between the N-terminal parts of the major envelope protein E of the yellow fever virus (17) and human pancreatic amylase. The alignment yielded 19.7 per cent sequence identity (standard deviation = 3.16), and 40.9 per cent conservative amino-acid substitutions according to the *Dayhoff* matrix (20) in a 119 amino-acid overlap. In figure 1 the aligned region of the yellow fever virus major envelope protein E (genome polyprotein-yellow fever virus, GNWVYP, 286 to 404, gene sequence numbering) is shown with the corresponding amino-acid residues in human pancreatic amylase (HUMAMY), porcine pancreatic amylase (PORAMY), and Taka amylase A (TAKAMY). The structural elements corresponding to the latter two sequences are given in lower case letters below the sequences (α: α-helix; g: 3 – 10 helix; β: β-strand). The alignment between the yeast and the mammalian amylase yielded an 18 per cent sequence identity and the sequences of human pancreatic and porcine pancreatic amylase an 85 per cent sequence identity.

At the positions where human pancreatic amylase and the yellow fever virus major envelope protein E were found to be identical, indicated by a straight vertical line, a colon or an asterisk in figure 1, the corresponding residues of Taka amylase A were examined to determine whether the position is likely to be buried or exposed. The structural analysis revealed that of the 23 identical amino-acid residues, 15 residues were exposed (straight vertical line), 6 residues buried (colon), and two residues were identified at positions where the structural homology between Taka amylase A and porcine pancreatic amylase is poor (asterisk).

The exposed amino-acid residues of Taka amylase A, corresponding to sequence identities between human pancreatic amylase and the yellow fever virus major envelope protein E, are indicated in the stereodrawing of the α-carbon backbone structure of the $(\alpha/\beta)_8$ barrel domain of Taka amylase A (fig. 2).

Discussion

In a recent review on macro enzymes *Remaley & Wilding* (2) drew attention to the question: "Why do antibodies to enzymes form?". It has been assumed that the "antigen-driven theory" and the "dysregulation of the immune tolerance theory" may partly explain their occurrence. We would like to suggest a further, related hypothesis that explains the published data: i. e. the mechanism of molecular mimicry, which could play an important role in the pathogenesis of autoimmune reactions if similar structures of an infectious agent and a host cell protein share antigenic sites (5). In this context, viruses in particular have already been discussed as a potential cause of autoimmunity (21). Among all of the known amino acid sequences, our investigation solely revealed one candidate bearing a region of significant sequence similarity with a fragment of human pancreatic amylase: i. e. the yellow fever virus major envelope protein E. This new information is supported by the following findings.

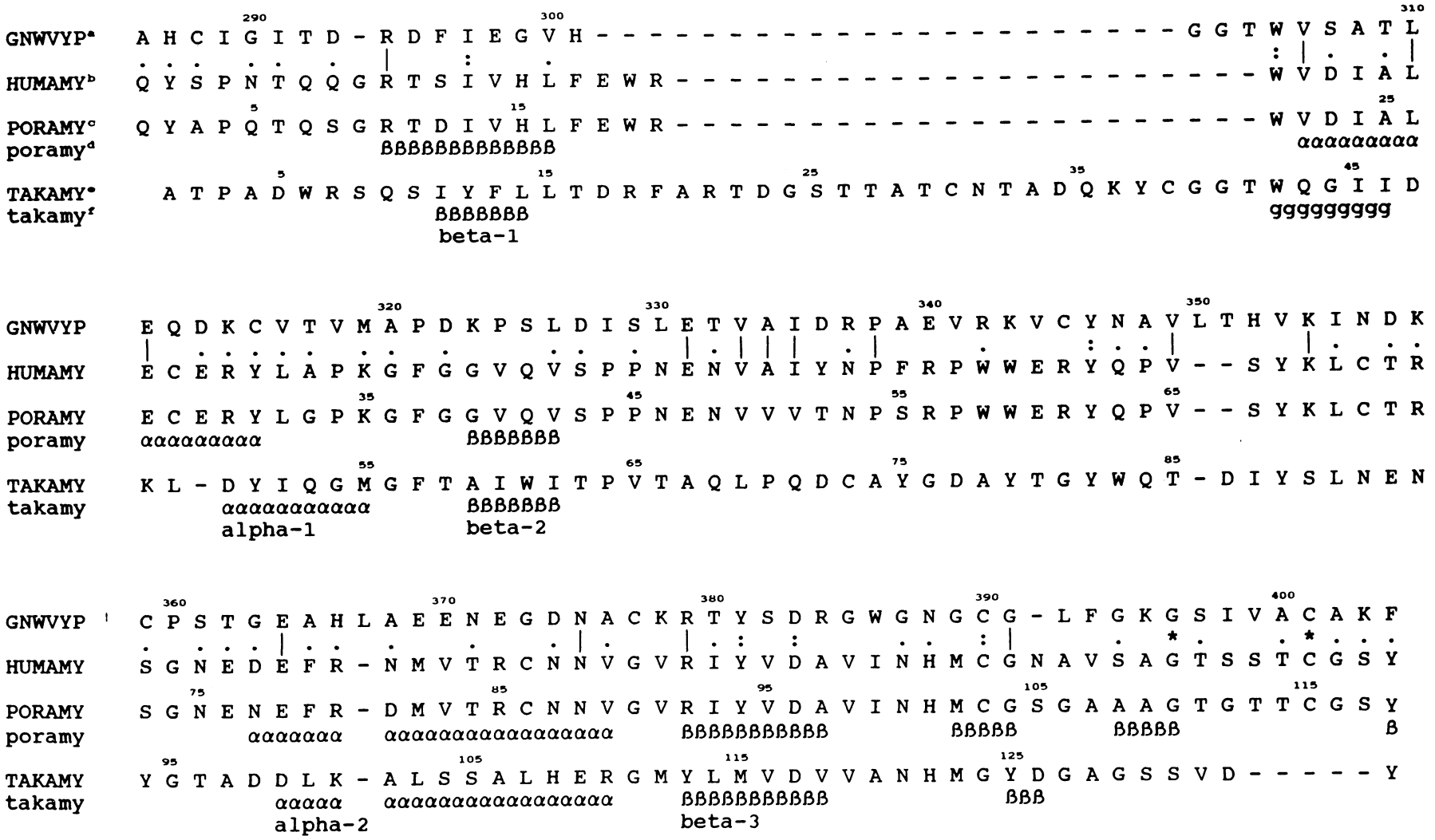

Fig. 1. Alignment of amylase sequences (single-letter amino-acid code) and the yellow fever virus major envelope protein E. Details are given in the main text.

[a] GNWVYP = yellow fever virus envelope protein
[b] HUMAMY = human pancreatic amylase
[c] PORAMY = porcine pancreatic amylase
[d] poramy = structural elements of porcine pancreatic amylase
[e] TAKAMY = Taka amylase A
[f] takamy = structural elements of Taka amylase A

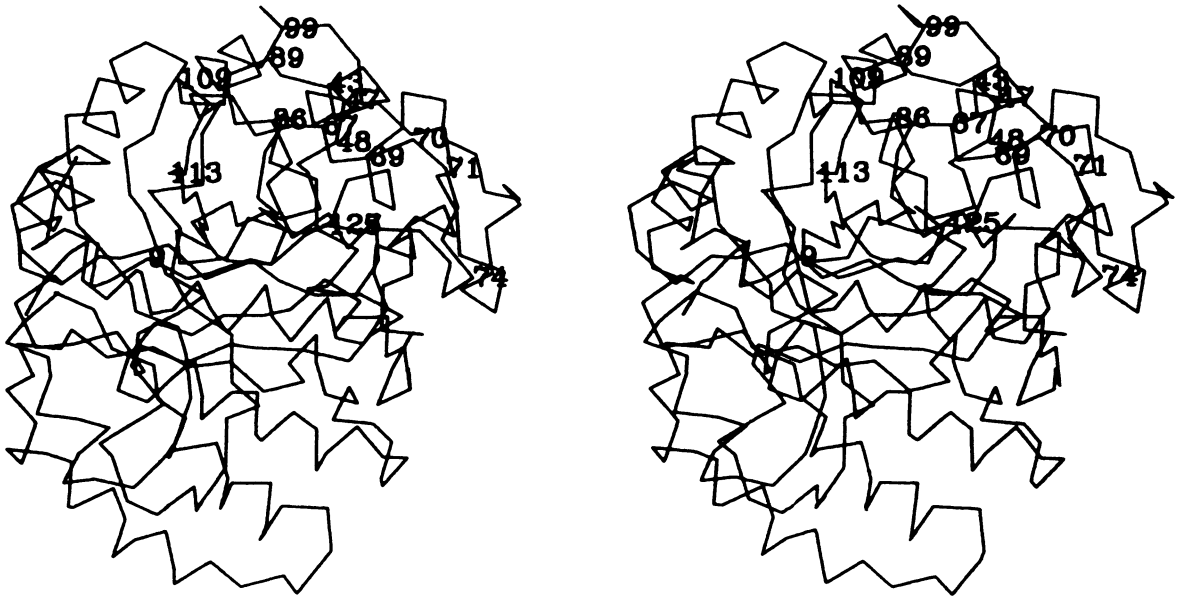

Fig. 2. Ribbon representation of the α-carbon backbone structure of the $(\alpha/\beta)_8$ barrel domain of Taka amylase A (residues 1 to 380). The positions of the 15 exposed amino-acid residues (indicated by a straight vertical line in fig. 1) corresponding to sequence identities between the yellow fever virus major envelope protein E and human pancreatic amylase are indicated by residue number labels. The drawing was generated using the program ARTIPLOT (16).

Inspection of the sequence of the entire RNA genome of the yellow fever virus reveals a single long open reading frame of 10233 nucleotides, which could encode a polypeptide of 3411 amino acids. The structural proteins are found within the amino-terminal 780 residues of this polyprotein. The sequence with a 19.5 per cent identity and a 40.9 per cent conservative amino-acid substitution over 119 amino acids is a part of the N-terminal sequence in the A domain of the major envelope protein E. This result supports the experimental data published by *Zinkernagel* et al. (22) using transgenic mice expressing the cell membrane associated glycoprotein of the vesicular stomatitis virus as a self-antigen. These transgenic mice could only be induced by the vesicular stomatitis virus to trigger autoantibodies if the self-antigen was a part of the viral envelope protein.

A distinguishing feature is the localization of antigenic determinants within the flavivirus envelope E protein structure. The B domain, a separate structural region of the E protein representing residues 309–403 and structurally dependent upon the location of the 315–345 disulphide bridge, contains the important antigenic determinants involved in haemagglutination and neutralization and thus induces immunological responses in the infected host (23).

Many viruses, particularly if persistent, are suspected to be involved in triggering autoantibodies (5). This persistent character is well documented by examples of cross-reacting immunoglobulins, which are stably maintained in the circulation of apparently healthy persons. For example, *Greco* (24) noted three cases of macro creatine kinase, type 1, stable over a period of at least two years; *Urdal* et al. (25) in their classical work reported a case in which macro amylase was still present three years after its initial discovery; and *Weijers* et al. (26) reported a case of macro lactate dehydrogenase, which to date is still present after its initial discovery in 1978.

A distinguishing feature of the above mentioned three "classical" immunoglobulin-complex macro enzymes is the light-chain restriction. The evidence of this study suggests that an infection with the yellow fever virus induces the observed phenomena. In this context, we would like to point out that autoantibodies to these enzymes do not appear to be curiosities, with no particular diagnostic or prognostic value, but that they reflect pathogenicity (27).

The first 400 or so amino-acid residues of both Taka amylase A and porcine amylase fold into a eight-stranded α/β barrel domain, a fold observed for 17 different enzyme structures so far, but no non-enzyme structures (28). The sequence homology found between the yellow fever virus major envelope protein E and human pancreatic amylase is limited to the first 119 amino-acid residues of the N-terminal sequence of the latter. From the known folding pattern of Taka amylase A, it was possible to identify 15 exposed

positions corresponding to sequence identities between human pancreatic amylase and the yellow fever virus major envelope protein E. These exposed residues are not biased over the entire surface of the ribbon representation of Taka amylase A (fig. 2) but located in a definite region forming a discontinuous site of at least 15 amino-acid residues. Studies of protein antigenicity (29) using three-dimensional X-ray crystallographic data from antigen-antibody complexes indicate that an epitope site consists of approximately 15 amino-acid residues on the surface of the antigen which are in contact with the binding site of the antibody molecule. Furthermore, we assume that the definite region of the yellow fever virus envelope protein and the amylase has a similar 3d fold. The 315–345 disulphide bridge of the envelope protein resembles the 28–86 disulphide bridge of human amylase. This would support the notion that the two regions have a locally similar tertiary structure.

The 119 amino-terminal amino acids of human pancreatic amylase and human salivary amylase are identical, except for position 4 (Pro → Ser) and position 52 (Tyr → His). These two positions do not participate in the sequence identities between human pancreatic amylase and the yellow fever virus major envelope protein E. It is therefore not surprising that most auto-antibodies of macro amylase complexes have little specificity for either type of amylase (25, 30–32).

The active site of the enzymes containing the typical eight-stranded α/β barrel structure is located in a cleft within the N-terminal central domain, at the carboxy-end of the β-strands of the $(\alpha/\beta)_8$ barrel (8, 28). In the molecular model a Taka amylase A the active site is located after the third β-strand (7). Thus, the active site and the site (epitope) on amylase recognized by the anti-amylase antibody are clearly separated. Consistent with this observation are the data showing that the autoantibodies were non-inhibitory towards the amylase activity (33, 34).

Because of the limited number of sequences for viral proteins in the computer library, the probability that the correct protein has been sequenced is less than 10 percent. The assigned yellow fever virus is a member of the family of *Flaviviridae*. The flaviviruses are classified as a separate family based on the structure of their genomes and on the replication strategies of these viruses (35). The family consists at least 66 viruses, 29 of which have been associated with human disease: i.e. give rise to persistent infection and chronic illness. To further complicate matters, new members are being recognized. In 1988 the virus now called hepatitis C virus was identified. The viral nucleic acid is a 10000 nucleotide RNA which best resembles flaviviruses in its genome (36). As more information becomes available on viral proteins, we expect to find additional homologies. The homology presented in this paper might be considered as a first approximation to be involved in the field of the "classical" macro enzymes.

In accordance with this theory and as a means of extending the hypothesis, collaborative projects of systematic screening to identify specific viral antibodies and antigens in individuals suffering from one of these three macro enzymes are in progress.

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